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Tobler, M ; Schlupp, I

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Influence of black spot disease on shoaling behaviour in female western mosquitofish, *Gambusia affinis* (Poeciliidae, Teleostei)

Michael Tobler · Ingo Schlupp

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Abstract Parasites can fundamentally alter the cost–benefit ratio of living in a group, e.g. if infected individuals increase the predation risk of shoal mates. Here, the effect of an infection with a trematode, *Uvulifer* sp. (Diplostomatidae) on the shoaling behaviour of female western mosquitofish, *Gambusia affinis*, was investigated. The parasite examined causes a direct phenotypical change of the host by forming black spots on its body surface. When given a choice between a stimulus shoal and no shoal, we found shoaling tendencies to be significantly reduced in infected focal fish. In another experiment, we tested for association preferences relative to the infection status of the stimulus fish. Given the choice between an infected and a healthy stimulus fish, both infected and healthy focal fish preferred to associate with non-infected stimulus fish. Our results suggest that (1) the cost–benefit ratio of shoaling might be different for infected and non-infected individuals. Infected fish may be more affected by competition for food within a shoal. (2) Associating with infected conspecifics appears

to be costly for female mosquitofish, maybe due to increased predation risk.

Keywords Group living · Predation · Parasites · *Uvulifer* · Fish behaviour

Introduction

Forming groups is a major antipredator adaptation in many prey species including fish (Helfman et al. 1997; Krause and Ruxton 2002). Antipredator benefits are maximized with increasing group size (higher vigilance and dilution effect) and increasing levels of coordination, regularity and phenotypic homogeneity within the group (Barber and Huntingford 1996). On the other hand, competition for resources increases with group size (Krause and Ruxton 2002). Factors influencing individual group choice therefore include group size and species composition and size of group members (Krause and Ruxton 2002).

Parasites can potentially affect costs and benefits of group living. For uninfected individuals, the probability to get infected increases with the formation of groups, if directly transmitted parasites are present (Dugatkin et al. 1994; Barber et al. 2000). Furthermore, the presence of infected fish may detract from the benefits of group living by reducing the levels of coordination, regularity and phenotypic homogeneity. For infected individuals,

M. Tobler (✉) · I. Schlupp
Department of Zoology, University of Oklahoma,
730 Van Vleet Oval, Norman, OK 73019, USA
e-mail: tobler@ou.edu

M. Tobler
Institute of Zoology, University of Zurich,
Winterthurerstr. 190, CH-8057 Zurich, Switzerland

living in a group may be disadvantageous, if parasitism increases the energy demand and/ or reduces competitive abilities (Barber et al. 2000).

In the present study, we examined the influence on fish shoaling behaviour of a parasite that directly affects the hosts' phenotype. Black spot disease (BSD) is caused by the metacercariae of a trematode, *Uvulifer* sp., which induce the production of black spots on the body surface of the fish (Bush et al. 2001). BSD is caused by a digenean trematode, *Uvulifer* sp., that relies on piscivorous birds as final host and water snails as first intermediate host to fulfil its life cycle (Lane and Morris 2000; Bush et al. 2001). Fish as second intermediate hosts are infected by free-swimming cercariae that penetrate the skin and become encysted metacercariae (Lane and Morris 2000). The metacercariae provoke the production of a fibrous capsule of host tissue around the parasite, which is followed by the migration of melanocytes into the cyst wall, creating the characteristic appearance of the black spot (Spellman and Johnson 1987, Bush et al. 2001). Parasite-induced black spots can easily be differentiated from other patterns formed by melanin containing cells (micro- and macro-melanophores) by their size, intensity and their random distribution on the body of the fish (Tobler, unpublished data). Consequently, the parasite induces a direct change of the host's phenotype and infected individuals are readily recognized by the presence of black spots. Based on the indirect life cycle, it is evident that BSD is not directly transmitted from fish to fish.

The influence of parasite-induced black spots on shoaling behaviour and shoal choice of fish has already been studied in other systems. Infected banded killifish, *Fundulus diaphanus* (LeSueur), have been demonstrated to show a reduced tendency to shoal (Krause and Godin 1994) and are avoided by conspecifics (Krause and Godin 1996). In another study, gynogenetic amazon mollies, *Poecilia formosa* (Girard), avoided video animations showing BSD-infected males of the sperm-donating sailfin, *P. latipinna* (Lesueur), and atlantic mollies, *P. mexicana* (Steindachner), however, this avoidance behaviour could not be found in females of *P. latipinna* and *P. mexicana* (Tobler et al. 2006).

In the present study, we investigated the influence of BSD on the shoaling behaviour of the poeciliid fish *Gambusia affinis* (western mosquitofish). Like other small poeciliid fishes, *G. affinis* experience strong predation by piscivorous fishes, snakes, birds and invertebrates and form shoals (Meffe and Snelson 1989; Pyke 2005). The present study focuses on two questions: (1) How does an infection with BSD affect the tendency to shoal in *G. affinis*? If BSD infection is linked to increased energy demands or reduced competitive abilities of the infected fish, it is expected that infected fish show reduced shoaling behaviour. (2) Does an infection with BSD affect association preferences relative to BSD infection? Infected fish may be avoided by both infected and non-infected conspecifics, if they are generally worse shoal mates (increasing the risk of predation). They may be preferred as shoal mates, if they are inferior competitors (reducing the cost of group formation). (3) Because BSD has a direct effect on the host's phenotype, phenotype-assortative shoaling may be predicted, whereby infected fish should associate with infected ones, whereas non-infected fish should prefer non-infected ones.

Materials and methods

Fish

Mosquitofish were caught in the San Marcos River (near Martindale, Central Texas) in June 2005 using a small seine. BSD has previously been recorded in the study population for several years and during all seasons in the study population (prevalence >10%; Tobler, unpublished data), suggesting that this parasite is potentially important in the study population. Fish were transported in aerated coolers with water from the collection sites to a greenhouse of the Aquatic Research Facility of the University of Oklahoma (Norman, Oklahoma), where they were maintained in 400-liter flow-through fibreglass tanks supplied with untreated well water. Naturally growing algae and mosquito larvae served as primary food source.

This diet was supplemented with commercially available fish food (TetraMin) three times a week.

Only females were used in this study to exclude any effect of sexual dimorphism, sexual attraction or sexual harassment (Pilastro et al. 2003; Dadda et al. 2005; Pyke 2005). This is not necessarily unnatural because sex ratios are—sometimes highly—biased towards females in natural populations of *G. affinis* (Snelson 1989; Tobler, unpublished data).

Experimental set-up

Shoaling experiments were performed in the greenhouse between 8:00 and 12:00. The test tank ($30 \times 76 \text{ cm}^2$ floor space) was visually divided into three equal sized sections by markings drawn on the front: a central neutral zone and two preference zones. In the centre of each preference zone, a transparent Plexiglas cylinder ($7.5 \times 7.5 \text{ cm}^2$ floor space) was positioned for the presentation of the stimuli. Hence, the focal fish could swim around the cylinder holding the stimuli. During the initial acclimatization period, another Plexiglas cylinder for the focal fish was positioned in the centre of the neutral zone. The back as well as the sides of the tank was covered with white polystyrene. Water level was maintained at 24 cm and water temperature was maintained between 23°C and 25°C. Fifty percent of the water was changed after every 10 trials and during the time when no experiment was running, the water was aerated and filtered.

Experiment 1: shoaling behaviour

To test for a reduced shoaling tendency in infected *G. affinis*, four non-infected stimulus females were haphazardly chosen from a stock tank and introduced in one of the two lateral Plexiglas cylinders. The cylinder in the other preference zone remained empty. Then a test female was haphazardly chosen from a stock tank and was placed into the Plexiglas cylinder in the neutral zone. The focal female was allowed to acclimate for 7 min. Then, the cylinder holding the test female was carefully removed and an observation period of five minutes followed. We

recorded the time spent in each preference zone. To detect side biases, the test female was immediately placed into the acclimatization cylinder again, the stimuli were interchanged and a second observation phase followed. After the test, the standard lengths of the focal females were measured to the closest millimetre and the number of black cysts on the focal fish was counted. Females were then transferred into another holding tank, so that each female was tested only once. However, due to limitations of the number of fish, the stimulus shoal was randomly composed from 29 pre-selected fish, so that individual fish were used as stimuli more than once, however, in different, random group combinations. All test females were only tested once. Neither stimulus nor focal fish were reused in experiment 2.

Experiment 2: association preferences

To test for association preferences relative to the infection status of the stimulus fish, a size-matched pair of stimulus fish, consisting of an infected and a non-infected female, was chosen from the stock tanks. Each fish was introduced into one of the two lateral cylinders. A focal female was then introduced into the central cylinder, and measurement of association times was conducted as described above. All fish were used only once in this experiment, either as stimulus or as test fish.

Statistical analyses

We decided a priori to exclude trials in which individual focal females spent more than 80% of the time during both parts of a trial in only one preference zone as side biases (Schlupp et al. 1994). Trials in which the focal females spent more than 50% of the time during both parts of a trial in the neutral zone were excluded as low responses (Schlupp et al. 1994). To test for female association preferences, the relative times spent near the shoal/the empty cylinder (experiment 1) or near the infected/non-infected stimulus fish (experiment 2) were compared using paired *t*-tests. Furthermore, the strength of preference (SOP) was calculated as: $(\text{time}_{\text{shoal/healthy}} - \text{time}_{\text{empty/BSD}}) / (\text{time}_{\text{shoal/healthy}} + \text{time}_{\text{empty/BSD}})$. SOP values can

vary between -1 (maximum avoidance of the stimulus) and $+1$ (maximum preference for the stimulus). SOPs were compared between infected and non-infected fish using independent t -tests. Alpha levels were corrected to account for multiple comparisons, whereby $\alpha' = 0.05/2 = 0.025$.

Results

Mean standard lengths and mean numbers of parasite-induced black spots for all females used are given in Table 1. Out of 34 trials in experiment 1, one was excluded from the analysis because of side bias. Both non-infected and BSD-infected females spent significantly more time in the preference zone with the group of conspecifics than in the empty preference zone (for non-

Table 1 Standard length (SL) and number of parasite induced black spots (BSD) for the females used in the different experiments. Means \pm SD are presented

		<i>N</i>	SL (mm)	# BSD
<i>Experiment 1</i>				
Test females	Healthy	15	36.6 ± 3.2	0.0 ± 0.0
	Infected	18	37.3 ± 3.7	15.7 ± 19.4
Stimulus females	Healthy	29	30.0 ± 1.1	0.0 ± 0.0
<i>Experiment 2</i>				
Test females	Healthy	22	38.6 ± 5.6	0.0 ± 0.0
	Infected	14	35.5 ± 3.3	14.0 ± 24.8
Stimulus females	Healthy	36	32.2 ± 3.1	0.0 ± 0.0
	Infected	36	32.3 ± 2.9	38.8 ± 21.2

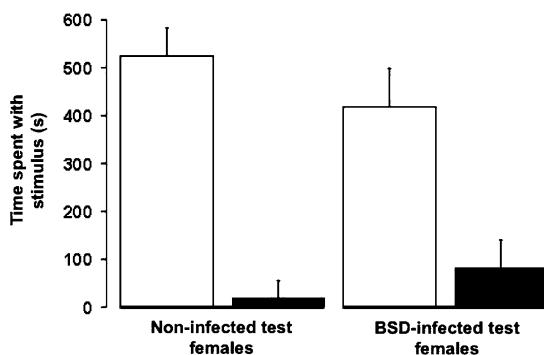


Fig. 1 Time spent with shoal (open bars) and time spent with empty cylinder (black bars) for non-infected (left; $N = 15$) and BSD-infected (right; $N = 18$) females. Both groups significantly preferred to associate with the shoal, but differed significantly in the strength of preference

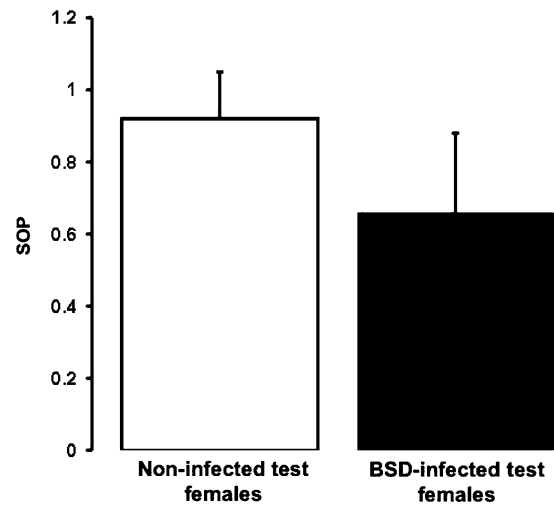


Fig. 2 BSD-infected females ($N = 18$) have a reduced tendency to shoal. Their strength of preference (SOP) was significantly lower compared to non-infected females ($N = 15$)

infected females: $t_{14} = 21.62$, $p < 0.001$, $\alpha' = 0.025$; for BSD-infected females: $t_{17} = 19.94$, $p < 0.001$, $\alpha' = 0.025$; Fig. 1). However, BSD-infected females exhibited significantly lower shoaling tendencies ($t_{32} = 4.06$, $p < 0.001$, $\alpha' = 0.025$; Fig. 2).

Out of 49 trials in experiment 2, eight were excluded from the analysis due to low response, five because of side biases. Both non-infected and BSD-infected females significantly preferred to associate with the non-infected stimulus (for non-infected females: $t_{21} = 3.20$, $p = 0.004$, $\alpha' = 0.025$; for BSD-infected females: $t_{13} = 3.46$, $p = 0.004$, $\alpha' = 0.025$; Fig. 3). There was no statistically significant difference in the SOP between infected and non-infected females ($t_{35} = 0.20$, $p = 0.84$, $\alpha' = 0.025$; for non-infected females, mean \pm SD: 0.36 ± 0.52 ; for BSD-infected females: 0.33 ± 0.36).

Discussion

Given a choice between a group of four conspecifics and no stimulus, both non-infected and BSD-infected females of *Gambusia affinis* preferred to associate with the shoal of conspe-

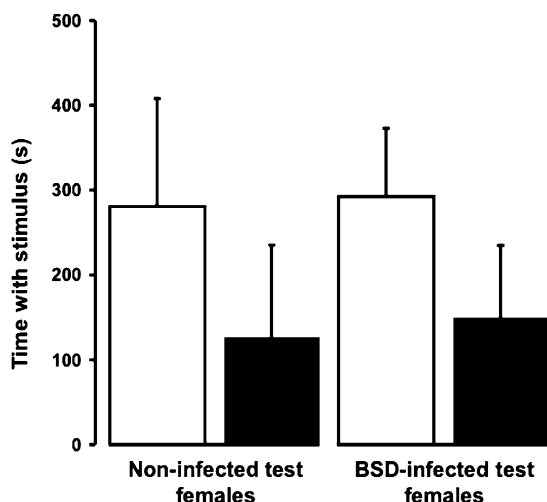


Fig. 3 Non-infected ($N = 22$) as well as infected ($N = 14$) females of *G. affinis* significantly preferred to associate with non-infected conspecifics (open bars) compared to BSD-infected ones (black bars)

cifics. However, the tendency to shoal was significantly reduced in BSD-infected females. Furthermore, *G. affinis* females preferred to associate with non-infected conspecifics irrespective of whether the females themselves were healthy or infected.

The reduced tendency to shoal in BSD-infected fish can be explained by a changed cost–benefit ratio of shoaling. Infected individuals may have higher energy demands. For example, it has been reported that an infection with BSD significantly increased the energy demand in bluegill sunfish, *Lepomis macrochirus* (Rafinesque), which was reflected in a negative correlation between body condition (measured as the ratio between weight and cubed standard length) and the number of black spots (Lemly and Esch 1984). The same negative correlation between body condition and BSD infection is also found in female *G. affinis* (Tobler, unpublished data). With increased energy demands, costs of group living in terms of food competition may be more relevant to infected *G. affinis*. Furthermore, the ability to compete for food with other shoal members may be reduced in BSD-infected fish as was shown in other fish-parasite systems (Crowden and Broom 1980 in dace parasitized by *Diplostomum*, Barber et al.

2000 for a review). Consequently, lower shoaling tendencies may be adaptive to infected fish to optimise foraging.

Although speculative, the reduced tendency to shoal in infected fish may be caused by manipulation by the parasite (Moore 2002). Any change in its intermediate host that increases the likelihood of this host being eaten by the final host—in the case of BSD piscivorous birds—is beneficial for a parasite (Lafferty 1999). By reducing the intermediate host's tendency to shoal, the parasite may increase the risk to be captured by its final host.

By reducing the tendency to shoal with non-infected conspecifics, infected females potentially avoid increased predation by the oddity effect, which describes the phenomenon of phenotypically distinct individuals within a shoal being more likely attacked by predators than phenotypically similar individuals (Landeau and Terborgh 1986; Theodorakis 1989; McRobert and Bradner 1998). However, both healthy and infected *G. affinis* females preferred to associate with non-infected conspecifics. This contradicts our prediction that infected females would prefer to associate with infected stimulus females to form phenotypically uniform shoals. Potentially, individual fish lack information regarding their own infection status.

Possibly, infected individuals are generally worse shoal mates that increase predation risk of shoal mates. Furthermore, associating with BSD-infected individuals may also increase the risk of being infected with other, directly transmitted diseases. Although BSD itself is not transmitted directly from fish to fish, infection with this parasite has been associated with secondary infections with other pathogens (Lane and Morris 2000). Future research, focusing on differences in the costs and benefits of shoaling behaviour between non-infected and BSD-infected *G. affinis* is highly warranted.

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